IN THE CLAIMS

The following listing of claims should be made of record and substituted for the current set of claims now in the application, with claims 1 and 3 shown as currently amended:

1. (Currently amended): A method for ~~evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides,~~ preparing a library of mutant polynucleotides, each mutant polynucleotide being prepared by a process comprising the steps of:

1) inserting [[a]] transposon Tn7 having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct, [[and]] cutting the transposon at the restriction enzyme sites to remove the transposon and ~~obtain~~ obtaining a linearized DNA construct containing two cut termini of the target DNA cut in one position;

2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one of the cut ~~terminus~~ termini of the target DNA;

3) inserting ~~a multiple of~~ three consecutive substitutive nucleotides into [[one]] the cut terminus of the target DNA subjected to deletion in Step 2, and deleting the nucleotides originating from the transposon and ~~a multiple of~~ three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1; and

4) subjecting both cut termini of the target DNA obtained in Step 3 to self-ligation to obtain ~~a library of mutant DNA~~ a mutant polynucleotide having substitutive nucleotides at a random position~~; and~~ of the target DNA.

5) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

2. (Original): The method of claim 1, wherein Step 2 comprises the steps of introducing a first cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting the cut terminus having nucleotides duplicated during the insertion of the transposon to a blunt end, thereby resulting in the deletion of the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon.

3. (Currently amended): The method of claim 1, wherein Step 3 comprises the steps of introducing a second cassette DNA containing ~~a multiple of~~ three consecutive substitutive nucleotides into the cut position of the DNA obtained in Step 2, digesting the second cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the addition of the substitutive nucleotides into [[one]] the cut terminus of the target DNA subjected to deletion in Step 2 and the deletion of the nucleotides originating from the transposon and ~~a multiple of~~ three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1.

4. (Original): The method of claim 1, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.

5. (Original): The method of claim 1, wherein the substitutive nucleotides introduced in Step 3 have a specific nucleotide sequence.

6. (Original): The method of claim 1, wherein the substitutive nucleotides introduced in Step 3 have a random nucleotide sequence.

7. (Previously presented): The method of claim 1, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

8. (Original): The method of claim 7, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.

9. (Previously presented): The method of claim 2, wherein the restriction enzyme is a class 11S restriction enzyme.

10. (Withdrawn): A method for evolving a polypeptide and a polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:

1) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the

restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

3) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step 1;

4) subjecting both cut tennini of the target DNA obtained in Step 3 to self-ligation to obtain a library of mutant DNA having additional nucleotides at a random position; and

5) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

11. (Withdrawn): The method of claim 10, wherein Step 2 comprises the steps of introducing a first cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting the cut terminus having nucleotides duplicated during the insertion of the transposon to a blunt end, thereby resulting in the deletion of the nucleotides originating from the

transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon.

12. (Withdrawn): The method of claim 10, wherein Step 3 comprises the steps of introducing a second cassette DNA containing a multiple of three consecutive additional nucleotides into the cut position of the DNA obtained in Step 2, digesting the second cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the insertion of the additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step 2, and the deletion of the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step 1.

13. (Withdrawn): The method of claim 10, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.

14. (Withdrawn): The method of claim 10, wherein the additional nucleotides introduced in Step 3 have a specific nucleotide sequence.

15. (Withdrawn): The method of claim 10, wherein the additional nucleotides introduced in Step 3 have a random nucleotide sequence.

16. (Withdrawn): The method of claim 10, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

17. (Withdrawn): The method of claim 16, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.

18. (Withdrawn): The method of claim 11, wherein the restriction enzyme is a class IIS restriction enzyme.

19. (Withdrawn): A method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:

1) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

2) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1;

3) subjecting both cut termini of the target DNA obtained in Step 2 to self-ligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and

4) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

20. (Withdrawn): The method of claim 19, wherein Step 2 comprises the steps of introducing a cassette DNA into the cut position of the target DNA, digesting the cassette DNA with a restriction enzyme, and converting both cut termini of the resulting DNA fragment to blunt ends, thereby resulting in the deletion of the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the deletion of the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step 1.

21. (Withdrawn): The method of claim 19, wherein the transposon is selected from the group consisting of Tn4430, Tn7, mini-Mu and derivatives thereof.

22. (Withdrawn): The method of claim 19, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

23. (Withdrawn): The method of claim 22, wherein the enzyme is selected from the group consisting of hydrolase, lyase, transferase, oxidoreductase, ligase and isomerase.

24. (Withdrawn): The method of claim 20, wherein the restriction enzyme is a class 11S restriction enzyme.

25. (Withdrawn): A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 1, into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

26. (Withdrawn): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 1 into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

27. (Previously presented): The method of claim 2, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

28. (Previously presented): The method of claim 3, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

29. (Previously presented): The method of claim 4, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

30. (Previously presented): The method of claim 5, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

31. (Previously presented): The method of claim 6, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

32. (Previously presented): The method of claim 3, wherein the restriction enzyme is a class IIS restriction enzyme.

33. (Previously presented): The method of claim 11, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

34. (Previously presented): The method of claim 12, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

35. (Previously presented): The method of claim 13, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

36. (Previously presented): The method of claim 14, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

37. (Previously presented): The method of claim 15, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

38. (Previously presented): The method of claim 12, wherein the restriction enzyme is a class IIS restriction enzyme.

39. (Previously presented): The method of claim 20, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

40. (Previously presented): The method of claim 21, wherein the target DNA encodes a protein selected from the group consisting of enzymes, antibodies, antigens, binding proteins, hormones, cytokines and plasma proteins.

41. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 10, into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

42. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more

mutant polynucleotides selected by the method of claim 19, into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same.

43. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 10 into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

44. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the

mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method of claim 19 into a target polynucleotide; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

45. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by a method for evolving a polypeptide and a polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having additional nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

46. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 1 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by a method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step a;

c) subjecting both cut termini of the target DNA obtained in Step b to self-ligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and

d) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

47. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having substitutive nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

48. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 10 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random deletion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA, and the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA at the other cut terminus of the target DNA obtained in Step a;

c) subjecting both cut termini of the target DNA obtained in Step b to self-ligation to obtain a library of mutant DNA having a deletion of nucleotides at a random position; and

d) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant

polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

49. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more mutant polynucleotides selected by the method for evolving a polypeptide and a polynucleotide encoding same by random substitution of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three substitutive nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon and a multiple of three consecutive nucleotides of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having substitutive nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.

50. (Previously presented): A method for evolving a polypeptide and a polynucleotide encoding same, comprising repeating the method of claim 19 with the mutant polynucleotide prepared by the method for evolving a polypeptide and a polynucleotide encoding same, comprising steps of:

1) preparing a library of mutant polynucleotides having a plurality of mutations by introducing two or more mutated sequences identified in two or more

mutant polynucleotides selected by a method for evolving a polypeptide and a polynucleotide encoding same by random insertion of nucleotides, comprising the steps of:

a) inserting a transposon having restriction enzyme sites on both ends thereof into a random position of a double-stranded target DNA, introducing the resulting DNA into a circular DNA construct and cutting the transposon at the restriction enzyme sites to remove the transposon and obtain a linearized DNA construct containing two cut termini of the target DNA cut in one position;

b) deleting the nucleotides originating from the transposon and the nucleotides of the target DNA duplicated during the insertion of the transposon, at one cut terminus of the target DNA;

c) inserting a multiple of three additional nucleotides into one cut terminus of the target DNA subjected to deletion in Step b, and deleting the nucleotides originating from the transposon at the other cut terminus of the target DNA obtained in Step a;

d) subjecting both cut termini of the target DNA obtained in Step c to self-ligation to obtain a library of mutant DNA having additional nucleotides at a random position; and

e) expressing the resulting library in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same; and

2) expressing the library obtained in Step 1 in an appropriate host cell and selecting or screening the expressed polypeptides to obtain a mutant polypeptide having a desired property and a polynucleotide encoding same as a target polynucleotide.